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Space Sciences Laboratory
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Semiannual Report

NASA Grant NGL 05-003-003

Task C

Analysis of Organic Matter in Sediments and Meteorites and
Paleochemical Studies of Extinct and Contemporary Life Forms

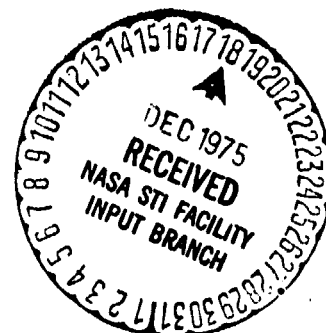
Principal Investigator

Professor M. Calvin

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Section I

Publications

Publications supported wholly or in part by NGL 05-003-003 during the period May 1, 1975 to October 31, 1975:

Kerogen--A Ubiquitous Source of Organic Carbon,
R.P. Philp, preprint, Scientific American, 1975.

Kerogenous Material in Recent Algal Mats at
Laguna Mormona, Baja California, R.P. Philp
and M. Calvin, Proc. 7th Intl. Meet. Organic
Geochemistry, Madrid, September 1975.

Kerogen Structures in Recently-Deposited Algal Mats
at Laguna Mormona, Baja California: A Model
System for the Determination of Kerogen Struc-
tures in Ancient Sediments, R.P. Philp and M.
Calvin, Proc. 2nd Environ. Biogeochem. Conf.,
Burlington, Canada, April 1975.

Copies of these publications are available upon request from Professor
M. Calvin.

Section II - Progress Report

TASK C. ANALYSIS OF ORGANIC MATTER IN SEDIMENTS AND METEORITES AND PALEOCHEMICAL STUDIES OF EXTINCT AND CONTEMPORARY LIFE FORMS

C1. Introduction

In the past year the main research effort of the Organic Geochemistry Group has been changing from the Mono Lake studies to a more detailed study of the insoluble organic material present in the algal mats at Laguna Mormona, Baja California. Detailed reports of the Mono Lake studies have appeared in our last two progress reports and a preliminary report of the Laguna Mormona study appeared in last year's report.

The Laguna Mormona study is the mainstay of our collaboration with the Organic Geochemistry Unit at the University of Bristol at the present time. The Bristol group has been examining the soluble lipid fractions and here at Berkeley we have been examining the insoluble residues remaining in these algal mat samples after exhaustive extraction. We have expanded our collaborative efforts to obtain some very valuable assistance from Dr. Stanley Awramik of the University of California at Santa Barbara. Dr. Awramik is a palaeontologist whose main interest is in Pre-Cambrian microfossils and their comparison with contemporary blue-green algae. He accompanied us on two field trips we made to Laguna Mormona during the past year and his expert advice in the field has been invaluable. We hope to continue our collaboration with him during the coming year.

The examination of the sterol content in the stratigraphic column from Mono Lake has been completed.

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A short study is now being performed on a series of six identical sediments collected from Mono Lake which have been stored under different conditions. The rationale behind this study was to see if any changes would be observed in the lipid distribution patterns as a result of differences in sample storage conditions. In many geochemical studies very little attention is paid to storage conditions and samples are frequently collected and allowed to sit at room temperature under aerobic conditions, whereas they may have been collected from an anaerobic environment at a much lower temperature. Although it is a tedious business, baseline studies such as these must be performed to get some idea about the variations to be expected when samples are stored under conditions different from those of their natural environment. Results from a similar study to this have been published previously, but it was a very limited study and the results were not very comprehensive.¹

In last year's report it was described how approximately seventy strains of bacteria had been isolated from some Recent Mono Lake sediments. Over the past year five of these strains have been cultured in bulk quantities and the sterol fractions from them have been isolated and analysed by the usual methods. Significant differences have been observed in the sterol content of these five strains. This adds further support to the utility of the sterols as a chemotaxonomical tool in distinguishing and classifying these particular bacteria, and perhaps the rest of the Monobacterium collection.

The work on finding more sophisticated reagents and techniques for the solubilization of kerogens from the Green River shale

kerogen has had to temporarily suspended due to the graduate student working on the project transferring to another group.

Finally the data system which is interfaced with our gas chromatograph-mass spectrometer has been further updated by the addition of a Versatec printer/plotter. This unit replaces the Tektronix hard copy unit installed with the original data system. It is anticipated that this will improve the standard of hard copy produced by the data system and will also be more economical to run.

C2. Accomplishments of Past Year and Current Status

(A) Degradation of the insoluble organic material isolated from the algal mats and oozes at Laguna Mormona, Baja California

Laguna Mormona offers an excellent site to study blue, green, and blue-green algae which are commonly regarded as ancient precursors of many higher life forms. The area contains examples of relatively recent algal mats which have been deposited over the past five hundred years. It is a discrete ecosystem, consisting predominantly of bacteria and algae living in an environment almost totally free from present-day pollution. It is essential that areas such as these are subjected to multi-disciplinary geochemical examinations for use as unpolluted baseline studies.

An examination of various geochemical aspects of samples from this area form the major part of the collaboration between the Berkeley and the Bristol groups. One of the major aims of this joint project is to obtain more detailed information about

the relationship between soluble lipid material and kerogen in this type of algal mat environment, and on a much longer time scale the possible mechanism of formation of petroleum in this type of environment.

The work on soluble lipid material is described elsewhere in this report and in this section we shall concentrate on the results from our degradation studies of the insoluble kerogen-like material present in these algal mats. The rationale for our part of the study is based on the fact that in many ancient sediments and oil shales a large part of the organic matter is derived from blue-green algae. Over a period of several million years the majority of this material has been incorporated into the insoluble kerogen fraction. It was anticipated that since the algal mats at Laguna Mormona are geologically very young any kerogen-like material present in the mats should be structurally less complex. Therefore by examining such samples, valuable information about the method of formation and the structure of kerogen should become available.

In last year's report, preliminary results were presented from a study of one sample of algal ooze obtained using a box coring device at a depth of 4 - 6". Since that time two other samples taken from immediately above that sample have been examined, providing us with a short geological sequence through the mats and oozes. These three samples of increasing age enable us to determine whether or not there are any changes in complexity of this insoluble material with increase in sample depth.

All three kerogen-like samples (S3.3, S3.2, and S3.1 --

S3.3 being the youngest and S3.1 the oldest sample) were isolated from the algal mats and oozes by treatment first with 6N HCl to remove any inorganic carbonates. Since it was found that these samples were predominantly carbonate, they were not further treated with hydrofluoric acid to remove any silicates which might have been present. The acid-treated samples were exhaustively extracted with toluene/methanol, toluene, and methanol for a total period of two weeks and subsequently dried under vacuo.

The residues obtained in this manner have so far been subjected to two types of degradation, i.e., oxidation and pyrolysis. It is anticipated that in the coming year they will be subjected to reduction using a variety of reagents.

(i) Oxidation

The residues from all three samples were degraded by chromic acid oxidation using the method Burlingame and Sinoneit² used in their stepwise degradation of kerogen isolated from the Green River shale. The results from our degradations are illustrated in Figures 1 and 2. Figure 1 shows the gas chromatograms of the total normal acid fractions (as methyl esters) obtained from the oxidation of the three samples. These acids were isolated from both the spent chromic acid and also the oxidized residue by heptane and ether extractions. The extracts were subsequently combined after they had been subjected to urea adduction. Figure 2 shows gas chromatograms of the heptane soluble branched/cyclic acids (as methyl esters) from the three oxidation mixtures. The ether soluble branched/cyclic acids were not examined by gas chromatography or combined gas chromatography-mass spectrometry

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due to the very low yields obtained of these fractions.

The acid fractions whose chromatograms are shown in Figure 1 are dominated by varying amounts of two series of normal acids. The fraction from S3.3 is dominated by a homologous series of monocarboxylic acids, in the range $C_{11} - C_{30}$ with a maximum at C_{16} . A series of α,ω -dicarboxylic acids is present in only minor amounts with a maximum at C_9 , in the range $C_7 - C_{20}$. However the fractions from S3.2 and S3.1 are both dominated by α,ω -dicarboxylic acids, with the maximum in the fraction from S3.2 being at C_8 and from S3.1 at C_9 . The fraction from S3.1 is more complex than either of the fractions from S3.2 or S3.3 with additional series of α -methyl monocarboxylic acids and mono-methyl dicarboxylic acids present in the mixtures.

The heptane soluble branched/cyclic acids, whose chromatograms are shown in Figure 2, do not show the same increase in complexity with increasing sample depth. The variations in distributions of the isoprenoid acids is clearly shown in these chromatograms. The origin of these isoprenoid acids is uncertain and we can only speculate on several alternative theories. They could have been trapped intact in the kerogen nucleus during its formation but partial degradation of the kerogen matrix by oxidation may have released these acids with their structures unchanged. Alternatively they may have been formed by random oxidation of carbon-carbon bonds, or they may have been ester-linked to the periphery of the kerogen. It has been shown in degradation studies of kerogen from the Green River shale³ that the stereochemistries of the isoprenoid acids formed are compatible with their being derived

from the side chain of chlorophyll. We have no proof at the present time to show that the situation is similar in the case of these algal mats. However it is noteworthy that the distribution of the isoprenoid acids in S3.3 is similar to that found in the Green River shale, with the maximum being a C_{16} .

In conclusion therefore it appears from these oxidative degradation studies that there is kerogen-like material in these algal mat deposits which has certain structural similarities to some ancient kerogens. The increase in complexity of the degradation products observed with increasing sample depth suggests that it may be possible to determine the initial reactions responsible for the kerogen formation by using this type of model system. From our results it appears that the basic framework of the kerogen consists of cross-linked polymethylene chains, with additional normal, isoprenoid and α -methyl branched acids attached to the periphery of the matrix.

(ii) Pyrolysis experiments

The kerogen-like residues isolated from the algal mats were also subjected pyrolytic degradations at varying temperatures and for varying periods of time. The amount of structural information obtained from this type of degradation study is minimal. It allows you to determine whether or not the material is predominantly aliphatic or aromatic in nature, but gives little information on the type of linkages between the various compounds which make up the basic kerogen matrix.

The pyrolysis experiments were performed over the temperature range $100-400^{\circ}$ and for time periods varying from 5 mins to 20

hours. The products were collected in toluene and after urea adduction, analysed by gas chromatography and combined gas chromatography-mass spectrometry. In the samples analysed to date the products have been dominated by series of n-alkanes and n-alkenes in the range C_5 to C_{20} with maximum at C_{11} and with no apparent odd-even predominance.

Although the work is still in its preliminary stages, as an initial observation it can be said that the products obtained are similar to those obtained from the kerogens of Precambrian samples such as the Fig Tree series. The products from our experiments were also almost exclusively aliphatic, again showing that algal kerogens have a predominantly aliphatic structure. In view of the similarity between these products and those of their ancient counterparts it is anticipated that a more detailed comparison between the results from the algal mats and Precambrian samples will lead to valuable information about the chemical nature of blue-green algae present in the Precambrian era.

(B) Mono Lake studies

The past year's studies of Mono Lake have taken two directions: first, a study of the effect of storage conditions on the sterol/stanol content of a recent mud, PI-W-2*; second, an examination of the sterol content of several strains of bacteria from our Monobacterium collection. These studies have been prompted by two features. First, until recently, little attention has been paid to the effect of storage conditions on the organic content of Recent muds, perhaps because it was believed that the diagenesis of source molecules is a long-term process.¹ Recent studies, however,

indicate that the process begins immediately on their incorporation into a mud.^{4,5} As has been shown in this study, storage conditions play a very important part in lipid distribution patterns. Second, our increasing interest in the contribution of Mono Lake's bacteria to the sediment's source sterols.

A Recent mud, PI-W-2*, collected 15.2 meters from Paoha Island in 1.2 meters of water was processed or stored under six different conditions, as outlined in Table 1. In the first three entries the muds were processed immediately to quench bacterial activity, while the last three samples were incubated for ten months, allowing bacterial diagenesis to proceed. The results reveal significant differences between the two groups. In the second group the total sterol pools are substantially lower, whereas the stanol/sterol ratios are higher. The higher ratios indicate that the unsaturated sterols are indeed being reduced, whereas the lower pools reveal that the molecules are also being transformed into other types of compounds. Closer examination of the second group also reveals that the pools are lower, and the stanol ratios higher, for those muds stored aerobically. Thus, the diagenesis, both into stanols and other molecules, apparently occurs preferentially under aerobic conditions.

Closer examination of the first group indicates that even the conditions of immediate processing and extraction will affect the organic content of the extract. Solvent extraction of the dried mud, coupled with sonication, seems to be more efficient in removing the total sterols, though the ratios are not appreciably affected.⁶ Finally, comparison of the mud stored

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in i-PrOH with the second group reveals that i-PrOH is effective in inhibiting diagenesis, presumably by quenching bacterial activity.

In light of the fact that more sterol diagenesis, which is an index of bacterial activity, occurred aerobically in the above study, we elected to culture the strains for our bacterial studies aerobically. Preliminary studies on a mixed culture of facultative anaerobes revealed the presence of several unsaturated sterols (Figure 3) indicating that the bacterial population of Mono Lake contributes to the pool of source sterols. Subsequently we selected five strains from our collection of facultative anaerobes and cultured them in bulk. After extraction and work-up, we obtained a sterol pool in three fractions: free sterols, easily extracted from the bacterial cells; esterified sterols, easily extracted but requiring alkaline hydrolysis after extraction; and finally bound sterols, bound to the cell debris and requiring alkaline hydrolysis prior to extraction. Examination of the results obtained to date in Table II reveals that Monobacteria do indeed contribute unsaturated sterols to the source pool.

As Table III illustrates, examination of the bound sterol fraction revealed the absence of any sterols in most of the strains. Fum a, however, contains a trace cholesterol. Cyt b is the only strain which contains quantitative levels of several species. Analysis of the sterol ester fraction is still in process, but preliminary results indicate the presence of sterols.

Evaluation of the results for the free sterol fractions of the various strains reveals substantial variations in the levels of sterols, the types found, and their relative abundance.

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Publications

1. Philp, R.P., Maxwell, J.R. and Eglinton, G. Environmental Organic Geochemistry. Submitted to Science Progress.
2. Toste, A.P., Steel, G., Philp, R.P. and Calvin, M. (1974). Incorporation and microbial diagenesis of sterols in Recent sediments. Presented at the 1974 Annual Meeting of the Geological Society of America, Miami, November 1974.
3. Cardoso, J., Brooks, P., Eglinton, G., Goodfellow, R., Maxwell, J.R. and Philp, R.P. Lipids in recently deposited algal mats at Mormona, Baja California. Presented at the Second International Symposium on Environmental Biogeochemistry in Ontario, Canada, April 1975. To be published in the Proceedings of this meeting.
4. Philp, R.P. and Calvin, M. Kerogen structures in recently-deposited algal mats at Laguna Mormona, Baja California: A model system for the determination of kerogen structures in ancient sediments. Presented at the Second International Symposium on Environmental Biogeochemistry in Ontario, Canada, April 1975. To be published in the Proceedings of this meeting.
5. Philp, R.P. and Calvin, M. Kerogenous material in Recent algal mats at Laguna Mormona, Baja California. Presented at the 7th International Meeting on Organic Geochemistry, Madrid, Spain, September 1975.
6. Philp, R.P. (1975). Kerogen -- A ubiquitous source of carbon. Accepted for publication in Scientific American.
7. Philp, R.P., Brown, S., and Calvin, M. (1975). Pyrolysis experiments on kerogen-precursors isolated from recently-

Task C (cont'd.)

Publications (cont'd.)

deposited algal oozes. To be presented at 1975 Geological Society of America meeting, Salt Lake City, Utah, October 1975.

8. Philp, R.P., Toste, A. and Calvin, M. (1975). Early stage formation and structure of kerogen-like material in Recent sediments. Submitted for presentation at 1975 ACS meeting, Los Angeles.

Table I.

Storage or processing conditions	Total ^a sterols (ppm)	C ₂₇ ^b ratio	C ₂₈ ^c ratio	C ₂₉ ^d ratio	total ^e ratio
<u>Group I</u>					
1. lyophilization (+ solvent extraction)	6.23	0.573	0.309	0.283	0.396
2. lyophilization (+ soxhlett extraction)	4.15	0.576	0.390	0.249	0.406
3. iPrOH addition (solvent extraction)	4.62	0.623	0.359	0.211	0.367
<u>Group II</u>					
4. aerobic incubation (10 months at room temp.) (solvent extraction)	1.53	0.815	0.704	0.358	0.550
5. aerobic incubation (10 months in room temp. water) (solvent extraction)	1.96	0.746	0.537	0.304	0.462
6. anaerobic incubation (10 months) (solvent extraction)	2.46	0.613	0.447	0.259	0.442

a: ppm expressed as the ratio: (total sterols/dry wt sediment) x 10⁶

b: ratio of C₂₇ stanols/total C₂₇ sterols: 5 α -cholestanol/5 α -cholestanol + cholesterol

c: ratio C₂₈ stanols/total C₂₈ sterols: 5 α -campestanol/5 α -campestanol + brassicasterol + campesterol

d: ratio of C₂₉ stanols/total C₂₉ sterol: 5 α -stigmastanol/5 α -stigmastanol + stigmasterol + β -sitosterol

e: ratio of total stanols/total sterols

Table II

<u>Monobacterium</u> <u>strain</u>	<u>Sterols^a</u>	<u>Total sterols^b</u> <u>(ppm)</u>
mannose a	cholesterol	3.68
	stigmasterol	
	β -sitosterol	
fumarate a	cholesterol	1.45
	β -sitosterol	
	unknown	
	campesterol	
cytosine b	cholesterol	0.97
	β -sitosterol	
	campesterol	
	brassicasterol	
	stigmasterol	
nicotinic acid a	cholesterol	0.22
	β -sitosterol	
glycine b	β -sitosterol	3.15
	cholesterol	
	campesterol	
	stigmasterol	
	unknown	
	brassicasterol (trace)	

a: the sterol components are listed in order of decreasing abundance, with the most abundant first.

b: ppm expressed as the ratio: (total sterols/dry wt bacteria) $\times 10^6$.

Task C (cont'd.)

Table III. Bound Sterol Fraction

Monobacterium strain	Sterols	Total sterols ^{a.} (ppm)
nicotine a	none	---
mannose a	none	---
fumarate a	cholesterol	trace
cytosine b	cholesterol	0.24
	stigmasterol	
	brassicasterol	

a. ppm expressed as the ratio: (total sterols/dry wt. sediment) x 10⁶

Figure 1. Gas chromatograms of the ether and heptane soluble normal acids (as methyl esters) from the 3 hour oxidation of the S3.2 residue. This clearly illustrates that ether extracts the more polar dicarboxylic acids and the heptane extracts the slightly higher molecular weight, less polar acids.

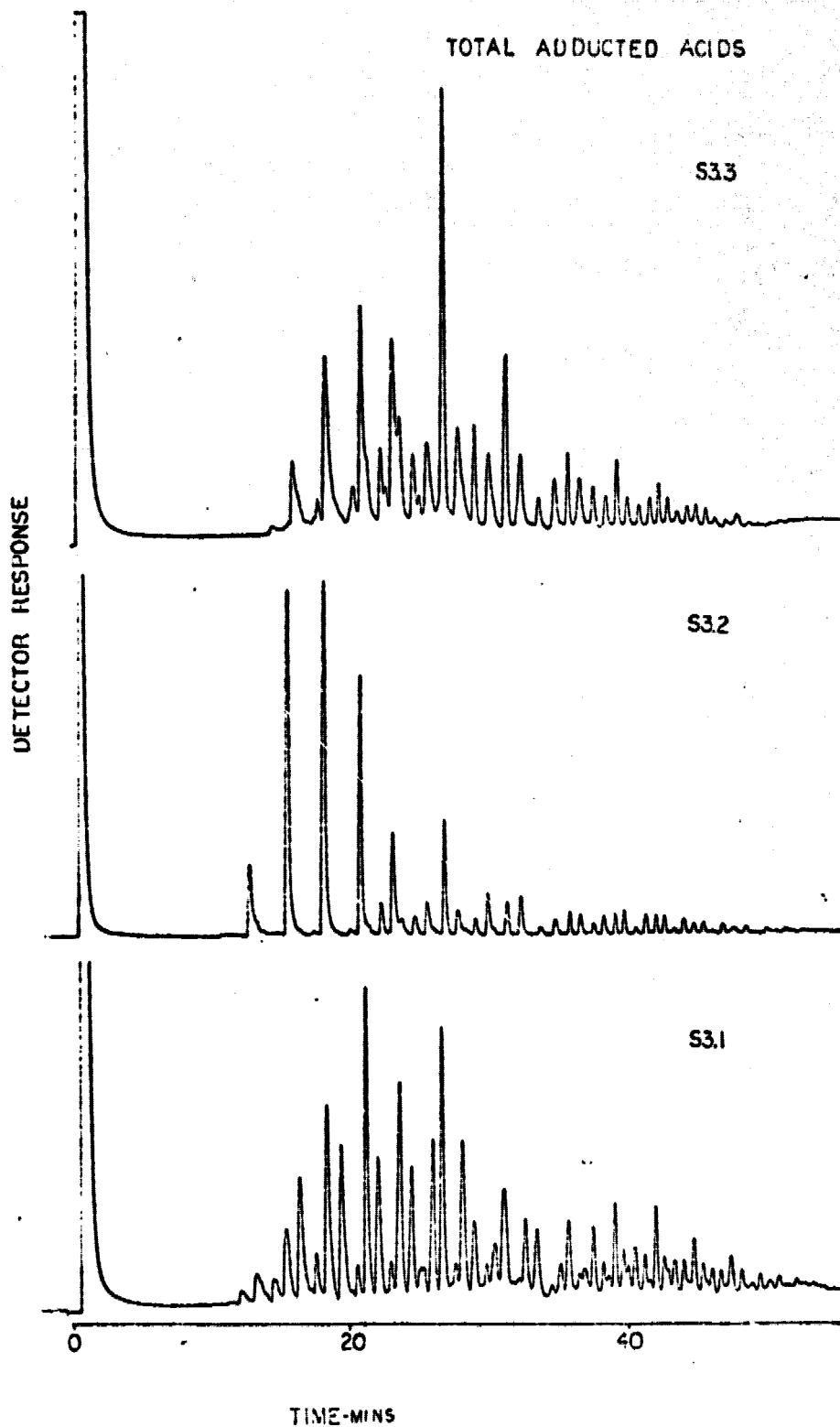
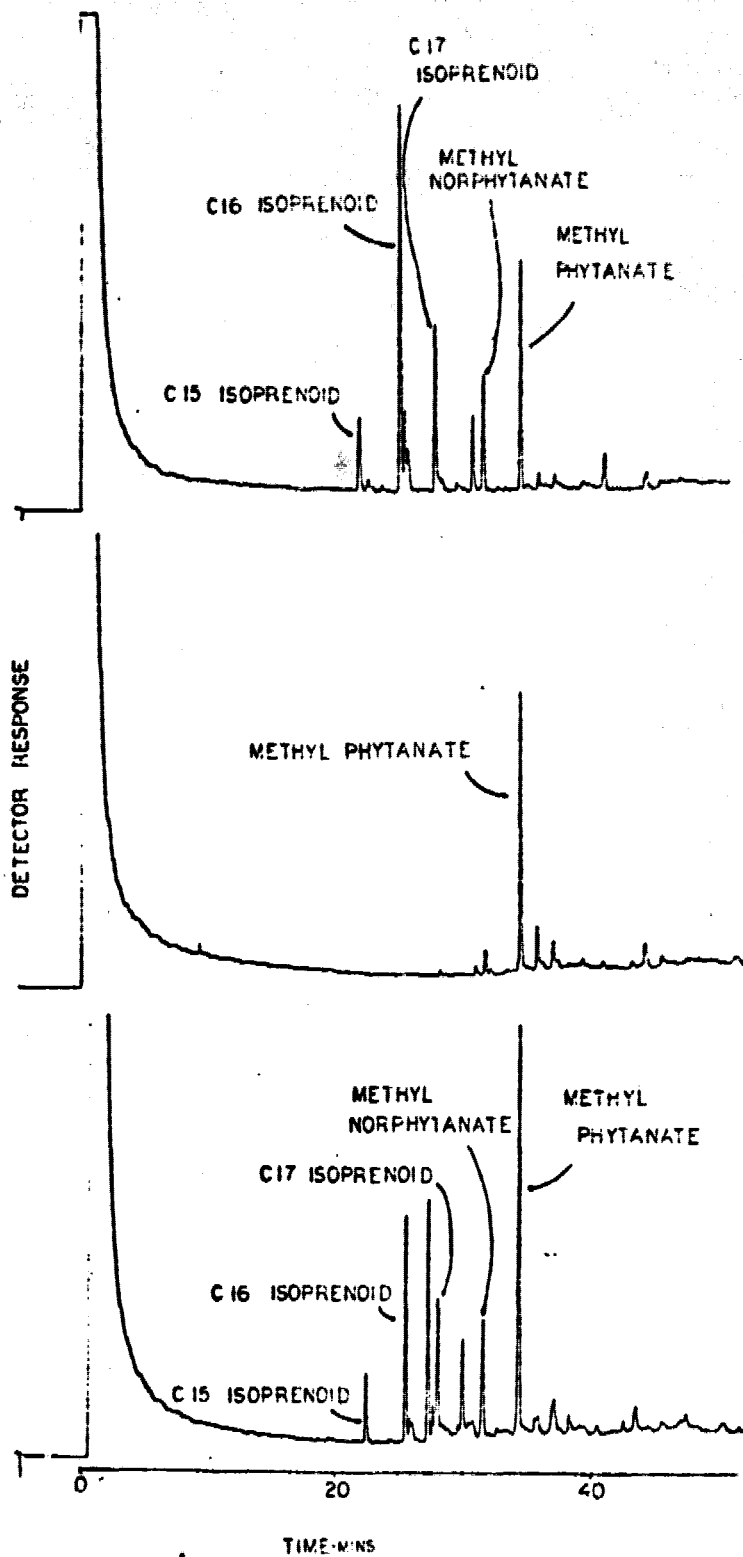


Figure 2. Gas chromatograms of the total normal or adducted acids (as methyl esters) from the 3 hour oxidations of the residues from S3.3, S3.2 and S3.1 respectively. S3.3 is dominated by $n\text{-C}_{16}$ and $n\text{-C}_{18}$ with only minor amounts of the α,ω -dicarboxylic acids. However the oxidation products from S3.2 and S3.1 are dominated by these dicarboxylic acids. The products from S3.1 are more complex than those from S3.2 with additional homologous series of α -methyl branched acids, which were not removed by urea adduction, and mono-methyl branched dicarboxylic acids.



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Task C (cont'd.)

Figure 3. Gas chromatogram of sterols from Mycobacteria.

